SECTION 6

FIELD PROCEDURES

This section provides guidance on sampling design of screening and intensive studies and recommends field procedures for collecting, preserving, and shipping samples to a processing laboratory for target analyte analysis. Planning and documentation of all field procedures are emphasized to ensure that collection activities are cost-effective and that sample integrity is preserved during all field activities.

6.1 SAMPLING DESIGN

Prior to initiating a screening or intensive study, the program manager and field sampling staff should develop a detailed sampling plan. As described in Section 2, there are seven major parameters that must be specified prior to the initiation of any field collection activities:

- Site selection
- Target species (and size class)
- Target analytes
- Target analyte screening values
- Sampling times
- Sample type
- Replicate samples.

In addition, personnel roles and responsibilities in all phases of the fish and shellfish sampling effort should be defined clearly. All aspects of the final sampling design for a State's fish and shellfish contaminant monitoring program should be documented clearly by the program manager in a Work/QA Project Plan (see Appendix F). Routine sample collection procedures should be prepared as standard operating procedures (U.S. EPA, 1984b) to document the specific methods used by the State and to facilitate assessment of final data quality and comparability.

The seven major parameters of the sampling plan should be documented on a sample request form prepared by the program manager for each sampling site. The sample request form should provide the field collection team with readily available information on the study objective, site location, site name/number, target species and alternate species to be collected, target analytes to be evaluated, anticipated sampling dates, sample type to be collected, number and size range of individuals to be collected for each composite sample, sampling

method to be used, and number of replicates to be collected. An example of a sample request form is shown in Figure 6-1. The original sample request form should be filed with the program manager and a copy kept with the field logbook.

The seven major parameters that must be specified in the sampling plan for screening and intensive studies are discussed in Sections 6.1.1 and 6.1.2, respectively.

6.1.1 Screening Studies (Tier 1)

The primary aim of screening studies is to identify frequently fished sites where commonly consumed fish and shellfish species are contaminated and may pose a risk to human health. Ideally, screening studies should include all waterbodies where commercial, recreational, or subsistence fishing and shellfish harvesting are practiced.

6.1.1.1 Site Selection—

Sampling sites should be selected to identify extremes of the bioaccumulation spectrum, ranging from presumed undisturbed reference sites to sites where existing data (or the presence of potential pollutant sources) suggest significant contamination. Where resources are limited, States initially should target those harvest sites suspected of having the highest levels of contamination and of posing the greatest potential health risk to local fish and shellfish consumers. Screening study sites should be located in frequently fished areas near

- Point source discharges such as
  - — Industrial or municipal dischargers
  - — Combined sewer overflows (CSOs)
  - — Urban storm drains
- Nonpoint source inputs such as
  - — Landfills, Resource Conservation and Recovery Act (RCRA) sites, or Superfund Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) sites
  - — Areas of intensive agricultural, silvicultural, or resource extraction activities or urban land development
  - — Areas receiving inputs through multimedia mechanisms such as hydrogeologic connections or atmospheric deposition (e.g., areas affected by acid rain impacts, particularly lakes with pH <6.0 since elevated mercury concentrations in fish have been reported for such sites)

Figure 6-1. Example of a sample request form.

- Areas acting as potential pollutant sinks where contaminated sediments accumulate and bioaccumulation potential might be enhanced (i.e., areas where water velocity slows and organic-rich sediments are deposited)

- Areas where sediments are disturbed by dredging activities

- Unpolluted areas that can serve as reference sites for subsequent intensive studies. For example, Michigan sampled lakes that were in presumed unpolluted areas but discovered mercury contamination in fish from many of these areas and subsequently issued a fish consumption advisory for all of its inland lakes.

The procedures required to identify candidate screening sites near significant point source discharges are usually straightforward. It is often more difficult, however, to identify clearly defined candidate sites in areas affected by pollutants from nonpoint sources. For these sites, assessment information summarized in State Section 305(b) reports should be reviewed before locations are selected. State 305(b) reports are submitted to the EPA Assessment and Watershed Protection Division biennially and provide an inventory of the water quality in each State. The 305(b) reports often contain Section 319 nonpoint source assessment information that may be useful in identifying major sources of nonpoint source pollution to State waters. States may also use a method for targeting pesticide hotspots in estuarine watersheds that employs pesticide use estimates from NOAA's National Coastal Pollutant Discharge Inventory (Farrow et al., 1989).

It is important for States to identify and document at least a few unpolluted sites, particularly for use as reference sites in subsequent monitoring studies. Verification that targeted reference sites show acceptably low concentrations of contaminants in fish or shellfish tissues also provides at least partial validation of the methods used to select potentially contaminated sites. Clear differences between the two types of sites support the site-selection methodology and the assumptions about primary sources of pollution.

In addition to the intensity of subsistence, sport, or commercial fishing, factors that should be evaluated (Versar, 1982) when selecting fish and shellfish sampling sites include

- Proximity to water and sediment sampling sites
- Availability of data on fish or shellfish community structure
- Bottom condition
- Type of sampling equipment
- Accessibility of the site.

The most important benefit of locating fish or shellfish sampling sites near sites selected for water and sediment sampling is the possibility of correlating contaminant concentrations in different environmental compartments (water, sediment, and fish). Selecting sampling sites in proximity to one another is also

more cost-effective in that it provides opportunities to combine sampling trips for different matrices.

Availability of data on the indigenous fish and shellfish communities should be considered in final site selection. Information on preferred feeding areas and migration patterns is valuable in locating populations of the target species (Versar, 1982). Knowledge of habitat preference provided by fisheries biologists or commercial fishermen may significantly reduce the time required to locate a suitable population of the target species at a given site.

Bottom condition is another site-specific factor that is closely related to the ecology of a target fish or shellfish population (Versar, 1982). For example, if only soft-bottom areas are available at an estuarine site, neither oysters (*Crassostrea virginica*) nor mussels (*Mytilus edulis* and *M. californianus*) would likely be present because these species prefer hard substrates. Bottom condition also must be considered in the selection and deployment of sampling equipment. Navigation charts provide depth contours and the locations of large underwater obstacles in coastal areas and larger navigable rivers. Sampling staff might also consult commercial fishermen familiar with the candidate site to identify areas where the target species congregates and the appropriate sampling equipment to use.

Another factor closely linked to equipment selection is the accessibility of the sampling site. For some small streams or land-locked lakes (particularly in mountainous areas), it is often impractical to use a boat (Versar, 1982). In such cases the sampling site should have good land access. If access to the site is by land, consideration should be given to the type of vegetation and local topography that could make transport of collection equipment difficult. If access to the sampling site is by water, consideration should be given to the location of boat ramps and marinas and the depth of water required to deploy the selected sampling gear efficiently and to operate the boat safely. Sampling equipment and use are discussed in detail in Section 6.2.1.

The selection of each sampling site must be based on the best professional judgment of the field sampling staff. Once the site has been selected, it should be plotted and numbered on the most accurate, up-to-date map available. Recent 7.5-minute (1:24,000 scale) maps from the U.S. Geologic Survey or blue line maps produced by the U.S. Army Corps of Engineers are of sufficient detail and accuracy for sample site mapping. The type of sampling to be conducted, water depth, and estimated time to the sampling site from an access point should be noted. The availability of landmarks for visual or range fixes should be determined for each site, and biological trawl paths (or other sampling gear transects) and navigational hazards should be indicated. Additional information on site-positioning methods, including Loran-C, VIEWNAV, TRANSIT (NAVSAT), GEOSTAR, and the NAVSTAR Global Positioning System (GPS), is provided in Battelle (1986), Tetra Tech (1986), and Puget Sound Estuary Program (1990a).

Each sampling site must be described accurately because State fish and shellfish contaminant monitoring data may be stored in a database available to users nationwide (see Section 9.2). For example, a sampling site may be defined as a 2-mile section of river (e.g., 1 mile upstream and 1 mile downstream of a reference point) or a 2-mile stretch of lake or estuarine/marine shoreline (U.S. EPA, 1990d). Each sampler should provide a detailed description of each site using a 7.5-minute USGS map to determine the exact latitude and longitude coordinates for the reference point of the site. This information should be documented on the sample request form and field record sheets (see Section 6.2.3).

6.1.1.2 Target Species and Size Class Selection—

After reviewing information on each sampling site, the field collection staff should identify the target species that are likely to be found at the site. Target species recommended for screening studies in freshwater systems are shown in Tables 3-1, 3-2, and 3-4. Tables 3-10 through 3-16 list recommended species for estuarine/marine areas. In freshwater ecosystems, one bottom-feeding and one predator fish species should be collected. In estuarine/marine ecosystems, either one bivalve species and one finfish species or two finfish species should be collected. Second and third choice target species should be selected in the event that the recommended target species are not collected at the site. The same criteria used to select the recommended target species (Section 3.2) should be used to select alternate target species. In all cases, the primary selection criterion should be that the target species is commonly consumed locally and is of harvestable size.

EPA recognizes that resource limitations may influence the sampling strategy selected by a State. If monitoring resources are severely limited, precluding performance of any Tier 2 intensive studies (Phase I and Phase II), EPA recommends three sampling options to States for collecting additional samples during the screening studies. These options are:

1. Collecting one composite sample for each of three size (age) classes of each target species

2. Collecting replicate composite samples for each target species

3. Collecting replicate composite samples for each of three size (age) classes of each target species.

Option 1 (single composite analysis for each of three size classes) provides additional information on size-specific levels of contamination that may allow States to issue an advisory for only the most contaminated size classes while allowing other size classes of the target species to remain open to fishing. The State could analyze the composite sample from the largest size class first. If any SVs are exceeded, analysis of the smaller size class composite samples could be conducted. This option, however, does not provide any additional

information for estimating the variability of the contamination level in any specific size class. To obtain information for estimating the variability of the contamination level in the target species, States could separately analyze each individual fish specimen in any composite that exceeded the SVs. **Note:** This option of analyzing individual fish within a composite sample is more resource-intensive with respect to analytical costs but is currently used by some Great Lakes States.

Option 2 (replicate analyses of one size class) provides additional statistical power that would allow States to estimate the variability of contamination levels within the one size class sampled; however, it does not provide information on size-specific contamination levels.

Option 3 (replicate analyses of three size classes) provides both additional information on size-specific contamination levels and additional statistical power to estimate the variability of the contaminant concentrations in each of three size classes of the target species. If resources are limited, the State could analyze the replicate samples for the largest size class first; if the SVs are exceeded, analysis of the smaller size class composite samples could then be conducted.

Note: The correlation between increasing size (age) and contaminant tissue concentration observed for some freshwater finfish species (Voiland et al., 1991) may be much less evident in estuarine/marine finfish species (G. Pollock, California Environmental Protection Agency, personal communication, 1993). The movement of estuarine and marine species from one niche to another as they mature may change their exposure at a contaminated site. Thus, size-based sampling in estuarine/marine systems should be conducted only when it is likely to serve a potential risk management outcome.

6.1.1.3 Target Analyte Selection—

All 25 recommended target analytes listed in Table 4-1 should be included in screening studies unless reliable historic tissue, sediment, or pollutant source data indicate that an analyte is not present at a level of concern for human health. Additional regional or site-specific target analytes should be included in screening studies when there is indication or concern that such contaminants are a potential health risk to local fish or shellfish consumers. Historic data on water, sediment, and tissue contamination and priority pollutant scans from known point source discharges or nonpoint source monitoring should be reviewed to determine whether analysis of additional analytes is warranted.

6.1.1.4 Target Analyte Screening Values—

To enhance national consistency in screening study data, States should use the target analyte screening values listed in Table 5-2 to evaluate tissue contaminant data. Specific methods used to calculate SVs for noncarcinogenic and carcinogenic target analytes, including examples of SVs calculated for selected subpopulations, are given in Sections 5.1 and 5.2. If target analytes in addition

to those recommended in Table 5-2 are included in a screening study, these calculation procedures should be used to estimate SVs based on typical exposure assumptions for the general population for the additional compounds. **Note:** If the State chooses to use a different risk level or consumption rate to address site-specific considerations, the corresponding SVs should be calculated prior to initiation of chemical analyses to ensure that the detection limits of the analytical procedures are sufficiently low to allow reliable quantitation at or below the chosen SV. If analytical methodology is not sensitive enough to reliably quantitate target analytes at or below selected SVs (see Section 8.2.2 and Table 8-4), program managers must determine appropriate fish consumption guidance based on lowest detectable concentrations or provide justification for adjusting SVs to values at or above achievable method detection limits. It should be emphasized that when SVs are below method detection limits, the failure to detect a target analyte can not be assumed to indicate that there is no cause for concern for human health effects.

6.1.1.5 Sampling Times—

If program resources are sufficient, biennial screening of waterbodies is recommended where commercial, recreational, or subsistence harvesting is commonly practiced (as identified by the State). Data from these screenings can then be used in the biennial State 305(b) reports to document the extent of support of Clean Water Act goals. If biennial screening is not possible, then waterbodies should be screened at least once every 5 years.

Selection of the most appropriate sampling period is very important, particularly when screening studies may be conducted only once every 2 to 5 years. **Note:** For screening studies, sampling should be conducted during the period when the target species is most frequently harvested (U.S. EPA, 1989d; Versar, 1982).

In fresh waters, as a general rule, the most desirable sampling period is from late summer to early fall (i.e., August to October) (Phillips, 1980; Versar, 1982). The lipid content of many species (which represents an important reservoir for organic pollutants) is generally highest at this time. Also, water levels are typically lower during this time, thus simplifying collection procedures. This late summer to early fall sampling period should not be used, however, if (1) it does not coincide with the legal harvest season of the target species or (2) the target species spawns during this period. **Note:** If the target species can be legally harvested during its spawning period, however, then sampling to determine contaminant concentrations should be conducted during this time.

A third exception to the late summer to early fall sampling recommendation concerns monitoring for the organophosphate pesticides. Sampling for these compounds should be conducted during late spring or early summer within 1 to 2 months following pesticide application because these compounds are degraded and metabolized relatively rapidly compared to organochlorine pesticides. **Note:** The target species should be sampled during the Spring only if the species can be legally harvested at this time.

In estuarine and coastal waters, the most appropriate sampling time is during the period when most fish are caught and consumed (usually summer for recreational and subsistence fishermen). For estuarine/marine shellfish (bivalve molluscs and crustaceans), two situations may exist. The legal harvesting season may be strictly controlled for fisheries resource management purposes or harvesting may be open year round. In the first situation, shellfish contaminant monitoring should be conducted during the legal harvest period. In the second situation, monitoring should be conducted to correspond to the period when the majority of harvesting is conducted during the legal season. State staff may have to consider different sampling times for target shellfish species if differences in the commercial and recreational harvesting period exist.

Ideally, the sampling period selected should avoid the spawning period of the target species, including the period 1 month before and 1 month after spawning, because many aquatic species are subject to stress during spawning. Tissue samples collected during this period may not always be representative of the normal population. For example, feeding habits, body fat (lipid) content, and respiration rates may change during spawning and may influence pollutant uptake and clearance. Collecting may also adversely affect some species, such as trout or bass, by damaging the spawning grounds. Most fishing regulations protect spawning periods to enhance propagation of important fishery species. Species-specific information on spawning periods and other life history factors is available in numerous sources (e.g., Carlander, 1969; Emmett et al., 1991; Pflieger, 1975; Phillips, 1980). In addition, digitized life history information is available in many States through the Multistate Fish and Wildlife Information System (1990).

Exceptions to the recommended sampling periods for freshwater and estuarine/marine habitats will be determined by important climatic, regional, or site-specific factors that favor alternative sampling periods. For many States, budgetary constraints may require that most sampling be conducted during June, July, and August when temporary help or student interns are available for hire. The actual sampling period and the rationale for its selection should be documented fully and the final data report should include an assessment of sampling period effects on the results.

6.1.1.6 Sample Type—

Composite samples of fish fillets or of the edible portions of shellfish are recommended for analysis of target analytes in screening studies (U.S. EPA, 1987b; 1989d). For health risk assessments, a composite sample should consist of that portion of the individual organism that is commonly consumed by the population at risk. Skin-on fillets (with the belly flap included) are recommended for most scaled finfish (see Sections 7.2.2.6 and 7.2.2.7). Other sample types (e.g., skinless fillets) may be more appropriate for some target species (e.g., catfish and other scaleless finfish species). For shellfish, the tissue considered to be edible will vary by target species (see Section 7.2.4.4) based on local food preferences. A precise description of the sample type (including the number and

size of the individuals in the composite) should be documented in the program records for each target species. **Note:** For freshwater turtles, the tissues considered to be edible vary based on the dietary and culinary practices of local populations (see Section 7.2.3.3). The EPA recommends use of individual turtle samples rather than composite samples for evaluating turtle tissue contamination.

Note: Composite samples are homogeneous mixtures of samples from two or more individual organisms of the same species collected at a particular site and analyzed as a single sample. Because the costs of performing individual chemical analyses are usually higher than the costs of sample collection and preparation, composite samples are most cost-effective for estimating average tissue concentrations of target analytes in target species populations. Besides being cost-effective, composite samples also ensure adequate sample mass to allow analyses for all recommended target analytes. A disadvantage of using composite samples, however, is that extreme contaminant concentration values for individual organisms are lost.

In screening studies, EPA recommends that States analyze one composite sample for each of two target species at each screening site. Organisms used in a composite sample

- Must all be of the same species
- Should satisfy any legal requirements of harvestable size or weight, or at least be of consumable size if no legal harvest requirements are in effect
- Should be of similar size so that the smallest individual in a composite is no less than 75 percent of the total length (size) of the largest individual
- Should be collected at the same time (i.e., collected as close to the same time as possible but no more than 1 week apart) [**Note:** This assumes that a sampling crew was unable to collect all fish needed to prepare the composite sample on the same day. If organisms used in the same composite are collected on different days (no more than 1 week apart), they should be processed within 24 hours as described in Section 7.2 except that individual fish may have to be filleted and frozen until all the fish to be included in the composite are delivered to the laboratory. At that time, the composite homogenate sample may be prepared.]
- Should be collected in sufficient numbers to provide a 200-g composite homogenate sample of edible tissue for analysis of recommended target analytes.

Individual organisms used in composite samples must be of the same species because of the significant species-specific bioaccumulation potential. Accurate taxonomic identification is essential in preventing the mixing of closely related species with the target species. **Note:** Under no circumstance should indivi-

duals from different species be used in a composite sample (U.S. EPA, 1989d, 1990d).

For cost-effectiveness, EPA recommends that States collect only one size class for each target species and focus on the larger individuals commonly harvested by the local population. Ideally, the individuals within each target species composite should be of similar size within a target size range. For persistent chlorinated organic compounds (e.g., DDT, PCBs, and toxaphene) and organic mercury compounds, the larger (older) individuals within a population are generally the most contaminated (Phillips, 1980; Voiland et al., 1991). As noted earlier, this correlation between increasing size and increasing contaminant concentration is most striking in freshwater finfish species but is less evident in estuarine and marine species. Size is used as a surrogate for age, which provides some estimate of the total time the individual organism has been at risk of exposure. Therefore, the primary target size range ideally should include the larger individuals harvested at each sampling site. In this way, the States will maximize their chances of detecting high levels of contamination in the single composite sample collected for each target species. If this ideal condition cannot be met, the field sampling team should retain individuals of similar length that fall within a secondary target size range.

Individual organisms used in composite samples should be of similar size (WDNR, 1988). **Note:** Ideally, for fish or shellfish, the total length (or size) of the smallest individual in any composite sample should be no less than 75 percent of the total length (or size) of the largest individual in the composite sample (U.S. EPA, 1990d). For example, if the largest fish is 200 mm, then the smallest individual included in the composite sample should be at least 150 mm. In the California Mussel Watch Program, a predetermined size range (55 to 65 mm) for the target bivalves (*Mytilus californianus and M. edulis*) is used as a sample selection criterion at all sampling sites to reduce size-related variability (Phillips, 1988). Similarly, the Texas Water Commission (1990) specifies the target size range for each of the recommended target fish species collected in the State's fish contaminant monitoring program.

Individual organisms used in a composite sample ideally should be collected at the same time so that temporal changes in contaminant concentrations associated with the reproduction cycle of the target species are minimized.

Each composite sample should contain 200 g of tissue so that sufficient material will be available for the analysis of recommended target analytes. A larger composite sample mass may be required when the number of target analytes is increased to address regional or site-specific concerns. However, the tissue mass may be reduced in the **Tier 2** intensive studies (Phase I and II) when a limited number of specific analytes of concern have been identified (see Section 7.2.2.9). Given the variability in size among target species, only approximate ranges can be suggested for the number of individual organisms to collect to achieve adequate mass in screening studies (U.S. EPA, 1989d; Versar, 1982). For fish, 3 to 10 individuals should be collected for a composite sample for each

target species; for shellfish, 3 to 50 individuals should be collected for a composite sample. In some cases, however, more than 50 small shellfish (e.g., mussels, shrimp, crayfish) may be needed to obtain the recommended 200-g sample mass. **Note:** The same number of individuals should be used in each composite sample for a given target species at each sampling site.

As alluded to above, one limitation of using composite samples is that information on extreme levels of contamination in individual organisms is lost. Therefore, EPA recommends that the residual individual homogenates be saved to allow for analyses of individual specimens if resources permit (Versar, 1982). Analysis of individual homogenates allows States to estimate the underlying population variance which, as described in Section 6.1.2.6, facilitates sample size determination for the intensive studies. Furthermore, individual homogenates may also be used to provide materials for split and spike samples for routine QC procedures either for composites or individual organisms (see Section 8.3). The circumstances in which the analysis of individual fish samples might be preferred over the analysis of composite samples is described in more detail in Appendix A.

Recommended sample preparation procedures are discussed in Section 7.2.

6.1.1.7 Replicate Samples—

The collection of sufficient numbers of individual organisms from a target species at a site to allow for the independent preparation of more than one composite sample (i.e., sample replicates) is strongly encouraged but is optional in screening studies. If resources and storage are available, single replicate (i.e., duplicate) composite samples should be collected at a minimum of 10 percent of the screening sites (U.S. EPA, 1990d). The collection and storage of replicate samples, even if not analyzed at the time due to inadequate resources, allow for followup QC checks. These sites should be identified during the planning phase and sample replication specifications noted on the sample request form. If replicate field samples are to be collected, States should follow the guidance provided in Section 6.1.2.7. **Note:** Additional replicates must be collected at each site for each target species if statistical comparisons with the target analyte SVs are required in the State monitoring programs. The statistical advantages of replicate sampling are discussed in detail in Section 6.1.2.7.

6.1.2 Intensive Studies (Tier 2)

The primary aim of intensive studies is to characterize the magnitude and geographic extent of contamination in harvestable fish and shellfish species at those screening sites where concentrations of target analytes in tissues were found to be above selected SVs. Intensive studies should be designed to verify results of the screening study, to identify specific fish and shellfish species and size classes for which advisories should be issued, and to determine the geographic extent of the fish contamination. In addition, intensive studies should be

designed to provide data for States to tailor their advisories based on the consumption habits or sensitivities of specific local human subpopulations.

State staff should plan the specific aspects of field collection activities for each intensive study site after a thorough review of the aims of intensive studies (Section 2.2) and the fish contaminant data obtained in the screening study. All the factors that influence sample collection activities should be considered and specific aspects of each should be documented clearly by the program manager on the sample request form for each site.

6.1.2.1 Site Selection—

Intensive studies should be conducted at all screening sites where the selected SV for one or more target analytes was exceeded. The field collection staff should review a 7.5-minute (1:24,000 scale) USGS hydrologic map of the study site and all relevant water, sediment, and tissue contaminant data. The site selection factors evaluated in the screening study (Section 6.1.1.1) must be reevaluated before initiating intensive study sampling.

States should conduct **Tier 2** intensive studies in two phases if program resources allow. **Phase I intensive studies** should be more extensive investigations of the magnitude of tissue contamination at suspect screening sites. **Phase II intensive studies** should define the geographic extent of the contamination around these suspect screening sites in a variety of size (age) classes for each target species. The field collection staff must evaluate the accessibility of these additional sites and develop a sampling strategy that is scientifically sound and practicable.

Selection of Phase II sites may be quite straightforward where the source of pollutant introduction is highly localized or if site-specific hydrologic features create a significant pollutant sink where contaminated sediments accumulate and the bioaccumulation potential might be enhanced (U.S. EPA, 1986f). For example, upstream and downstream water quality and sediment monitoring to bracket point source discharges, outfalls, and regulated disposal sites showing contaminants from surface runoff or leachate can often be used to characterize the geographic extent of the contaminated area. Within coves or small embayments where streams enter large lakes or estuaries, the geographic extent of contamination may also be characterized via multilocational sampling to bracket the areas of concern. Such sampling designs are clearly most effective where the target species are sedentary or of limited mobility (Gilbert, 1987). In addition, the existence of barriers to migration, such as dams, should be taken into consideration.

6.1.2.2 Target Species and Size Class Selection—

Whenever possible, the target species found in the screening study to have elevated tissue concentrations of one or more of the target analytes should be resampled in the intensive study. Recommended target species for freshwater

sites are listed in Tables 3-1, 3-2, and 3-4; target species for estuarine/marine waters are listed in Tables 3-10 through 3-12 for Atlantic Coast estuaries, in Table 3-13 for Gulf Coast estuaries, and in Tables 3-14 through 3-16 for Pacific Coast estuaries. If the target species used in the screening study are not collected in sufficient numbers, alternative target species should be selected using criteria provided in Section 3.2. The alternative target species should be specified on the sample request form.

For Phase I intensive studies, States should collect replicate composite samples of one size class for each target species and focus sampling on larger individuals commonly harvested by the local population (as appropriate). If contamination of this target size class is high, Phase II studies should include collection of replicate composite samples of three size classes within each target species.

EPA recognizes that resource limitations may influence the sampling strategy selected by a State. If monitoring resources are limited for intensive studies, States may determine that it is more resource-efficient to collect replicate composite samples of three size classes (as required for Phase II studies) during Phase I sampling rather than revisit the site at a later time to conduct Phase II intensive studies. In this way, the State may save resources by reducing field sampling costs associated with Phase II intensive studies.

By sampling three size (age) classes, States collect data on the target species that may provide them with additional risk management options. If contaminant concentrations are positively correlated with fish and shellfish size, frequent consumption of smaller (less contaminated) individuals may be acceptable even though consumption of larger individuals may be restricted by a consumption advisory. In this way, States can tailor an advisory to protect human health and still allow restricted use of the fishery resource. Many Great Lakes States have used size (age) class data to allow smaller individuals within a given target species to remain fishable while larger individuals are placed under an advisory.

6.1.2.3 Target Analyte Selection—

Phase I intensive studies should include only those target analytes found in the screening study to be present in fish and shellfish tissue at concentrations exceeding selected SVs (Section 5.2). Phase II studies should include only those target analytes found in Phase I intensive studies to be present at concentrations exceeding SVs. In most cases, the number of target analytes evaluated in Phase I and II intensive studies will be significantly smaller than the number evaluated in screening studies.

6.1.2.4 Target Analyte Screening Values—

Target analyte SVs used in screening studies should also be used in Phase I and II intensive studies. Specific methods used to calculate SVs for noncarcinogenic and carcinogenic target analytes, including examples of SVs calculated for various exposure scenarios, are given in Section 5.1.

6.1.2.5 Sampling Times—

To the extent that program resources allow, sampling in intensive studies should be conducted during the same period or periods during which screening studies were conducted (i.e., when the target species are most frequently harvested for consumption) and should be conducted preferably within 1 year of the screening studies. In some cases, it may be best to combine Phase I and Phase II sampling to decrease both the time required to obtain adequate data for issuance of specific advice relative to species, size classes, and geographic extent and/or the monitoring costs entailed in revisiting the site (see Section 6.1.2.2).

States should follow the general guidance provided in Section 6.1.1.5 for recommended sampling times. The actual sampling period and rationale for its selection should be documented fully for Phase I and II studies.

6.1.2.6 Sample Type—

Composite samples of fish fillets or the edible portions of shellfish are recommended for analysis of target analytes in intensive studies. The general guidance in Section 6.1.1.6 should be followed to prepare composite samples for each target species. In addition, separate composite samples may be prepared for selected size (age) classes within each target species, particularly in Phase II studies after tissue contamination has been verified in Phase I studies. Because the number of replicate composite samples and the number of fish and shellfish per composite required to test whether the site-specific mean contaminant concentration exceeds an SV are intimately related, both will be discussed in the next section.

Note: The same number of individual organisms should be used to prepare all replicate composite samples for a given target species at a given site. If this number is outside the recommended range, documentation should be provided.

Recommended sample preparation procedures are discussed in Section 7.2.

States interested in analyzing target analyte residues in individual fish or shellfish samples should review information presented in Appendix A.

6.1.2.7 Replicate Samples—

In intensive studies (Phases I and II), EPA recommends that States analyze replicate composite samples of each target species at each sampling site.

Replicate composite samples should be as similar to each other as possible. In addition to being members of the same species, individuals within each composite should be of similar length (size) (see Section 6.1.1.6). The relative difference between the average length (size) of individuals within any composite sample from a given site and the average of the average lengths (sizes) of

individuals in all composite samples from that site should not exceed 10 percent (U.S. EPA, 1990d). In order to determine this, States should first calculate the average length of the target species fish constituting each composite replicate sample from a site. Then, States should take the average of these averages for the site. In the following example, the average of the average lengths of individuals (±10 percent) in five replicate composite samples is calculated to be 310 (±31) mm.

Replicate	Average Length of Individual Fish in Composite Sample (mm)
1	300
2	320
3	330
4	280
5	320

Average of the average length (±10%) = 310 (±31) mm.

Therefore, the acceptable range for the average length of individual composite samples is 279 to 341 mm, and the average length of individual fish in each of the five replicate composites shown above falls within the acceptable average size range.

All replicate composite samples for a given sampling site should be collected within no more than 1 week of each other so that temporal changes in target analyte concentrations associated with the reproductive cycle of the target species are minimized.

The remainder of this section provides general guidelines for estimating the number of replicate composite samples per site (n) and the number of individuals per composite (m) required to test the null hypothesis that the mean target analyte concentration of replicate composite samples at a site is equal to the SV versus the alternative hypothesis that the mean target analyte concentration is greater than the SV. These guidelines are applicable to any target species and any target analyte.

Note: It is not possible to recommend a single set of sample size requirements (e.g., number of replicate composite samples per site and the number of individuals per composite sample) for all fish and shellfish contaminant monitoring studies. Rather, EPA presents a more general approach to sample size determination that is both scientifically defensible and cost-effective. At each site, States must determine the appropriate number of replicate composite samples and of individuals per composite sample based on

- Site-specific estimations of the population variance of the target analyte concentration

- Fisheries management considerations
- Statistical power consideration.

If the population variance of the target analyte concentrations at a site is small, fewer replicate composite samples and/or fewer individuals per composite sample may be required to test the null hypothesis of interest with the desired statistical power. In this case, using sample sizes that are larger than required to achieve the desired statistical power would not be cost-effective.

Alternatively, suppose EPA recommended sample sizes based on an analyte concentration with a population variance that is smaller than that of the target analyte. In this case, the EPA-recommended sample size requirements may be inadequate to test the null hypothesis of interest at the statistical power level selected by the State. Therefore, EPA recommends an approach that provides the flexibility to sample less in those waters where the target analyte concentrations are less variable, thereby reserving sampling resources for those site-specific situations where the population variance of the target analyte tissue concentration is greater.

The EPA recommends the following statistical model, which assumes that z_i is the contaminant concentration of the ith replicate composite sample at the site of interest where i=1,2,3,...,n and, furthermore, that each replicate composite sample is comprised of m individual fish fillets of equal mass. Let $\bar{z}$ be the mean target analyte concentration of observed replicate composite samples at a site. Ignoring measurement error, the variance of $\bar{z}$ is

$$\text{Var}(\bar{z}) = \sigma^2/(nm) \qquad (6\text{-}1)$$

where

σ^2 = Population variance
n = Number of replicate composite samples
m = Number of individual samples in each composite sample.

To test the null hypothesis that the mean target analyte concentration across the n replicate composite samples is equal to the SV versus the alternative hypothesis that the mean target analyte concentration is greater than the SV, the estimate of the $\text{Var}(\bar{z})$, s^2, is

$$s^2 = [\Sigma(z_i - \bar{z})^2] / [n(n - 1)] \qquad (6\text{-}2)$$

where the summation occurs over the n composite samples. Under the null hypothesis, the following statistic

$$(\bar{z} - SV) / s \qquad (6\text{-}3)$$

has a Student-t distribution with (n − 1) degrees of freedom (Cochran, 1977; Kish, 1965). The degrees of freedom are one less than the number of composite samples.

An optimal sampling design would specify the minimum number of replicate composite samples (n) and of individuals per composite (m) required to detect a minimum difference between the SV and the mean target analyte concentration of replicate composite samples at a site. Design characteristics necessary to estimate the optimal sampling design include

- Minimum detectable difference between the site-specific mean target analyte concentration and the SV
- Power of the hypothesis test (i.e., the probability of detecting a true difference when one exists)
- Level of significance (i.e., the probability of rejecting the null hypothesis of no difference between the site-specific mean target analyte concentration and the SV when a difference does not exist)
- Population variance, σ^2 (i.e., the variance in target analyte concentrations among individuals from the same species, which the statistician often must estimate from prior information)
- Cost components (including fixed costs and variable sample collection, preparation, and analysis costs).

In the absence of such design specifications, guidance for selecting the number of replicate composite samples at each site and the number of fish per composite sample is provided. This guidance is based on an investigation of the precision of the estimate of σ^2/nm and of statistical power.

Note: Under optimal field and laboratory conditions, at least two replicate composite samples are required at each site for variance estimation. To minimize the risk of a destroyed or contaminated composite sample precluding the site-specific statistical analysis, a minimum of three replicate composite samples should be collected at each site if possible. Because three replicate composite samples provide only two degrees of freedom for hypothesis testing, additional replicate composite samples are recommended.

The stability of the estimated standard error of $\bar{z}$ must also be considered because this estimated standard error is the denominator of the statistic for testing the null hypothesis of interest. A measure of the stability of an estimate is its statistical precision. The assumption is made that the z_i's come from a normal distribution, and then the standard error of $\hat{\sigma}^2/nm$ is defined as a product of σ^2 and a function of n (the number of replicate composite samples) and m (the number of fish per composite). A fortunate aspect of composite sampling is that the composite target analyte concentrations tend to be normally

distributed via the Central Limit Theorem. This formulation is used to determine which combinations of n and m are associated with a more precise estimate of σ^2/nm.

Modifying Cochran (1963) to reflect the normality assumption and the sampling design of n replicate composite samples and m fish per composite sample, the function of n and m of interest is shown in square brackets:

$$se\left(\frac{\hat{\sigma}^2}{nm}\right) = \sigma^2\left[\frac{2}{n^2m^2(n-1)}\right]^{1/2} \qquad (6\text{-}4)$$

Table 6-1 provides values of this function for various combinations of m and n. The data presented in Table 6-1 suggest that, as either n or m increases, the standard error of $\hat{\sigma}^2/nm$ decreases. The advantage of increasing the number of replicate composite samples can be described in terms of this standard error. For example, the standard error of $\hat{\sigma}^2/nm$ from a sample design of five replicate composite samples and six fish per composite (0.024) will be more than 50 percent smaller than that from a sample design of three replicate composite samples and six fish per composite (0.056). In general, holding the number of fish per composite fixed, the standard error of $\hat{\sigma}^2/nm$ estimated from five replicate samples will be about 50 percent smaller than that estimated from three replicate samples.

Table 6-1. Values of $\left[\frac{2}{n^2m^2(n-1)}\right]^{1/2}$ for Various Combinations of n and m

No. of replicate composite samples (n)	Number of fish per composite sample (m)									
	3	4	5	6	7	8	9	10	12	15
3	0.111	0.083	0.067	0.056	0.048	0.042	0.037	0.033	0.028	0.022
4	0.068	0.051	0.041	0.034	0.029	0.026	0.023	0.020	0.017	0.014
5	0.047	0.035	0.028	0.024	0.020	0.018	0.016	0.014	0.012	0.009
6	0.035	0.026	0.021	0.018	0.015	0.013	0.012	0.011	0.009	0.007
7	0.027	0.021	0.016	0.014	0.012	0.010	0.009	0.008	0.007	0.005
10	0.016	0.012	0.009	0.008	0.007	0.006	0.005	0.005	0.004	0.003
15	0.008	0.006	0.005	0.004	0.004	0.003	0.003	0.003	0.002	0.002

The data in Table 6-1 also suggest that greater precision in the estimated standard error of $\overline{z}$ is gained by increasing the number of replicate samples (n) than by increasing the number of fish per composite (m). If the total number of individual fish caught at a site, for example, is fixed at 50 fish, then, with a design of 10 replicate samples of 5 fish each, the value of the function of n and m in Table 6-1 is 0.009; with 5 replicate samples of 10 fish each, the value is 0.014. Thus, there is greater precision in the estimated standard error of $\overline{z}$ associated with the first design as compared with the second design.

Two assumptions are made to examine the statistical power of the test of the null hypothesis of interest. First, it is assumed that the true mean of the site-specific composite target analyte concentrations (μ) is either 10 percent or 50 percent higher than the screening value. Second, it is presumed that a factor similar to a coefficient of variation, the ratio of the estimated population standard deviation to the screening value (i.e., σ/SV), is 50 to 100 percent. Four scenarios result from joint consideration of these two assumptions. The power of the test of the null hypothesis that the mean composite target analyte concentration at a site is equal to the SV versus the alternative hypothesis that the mean target analyte concentration is greater than the SV is estimated under each set of assumptions. Estimates of the statistical power for two of the four scenarios are shown in Table 6-2.

Power estimates for the two scenarios where the true mean of the site-specific composite target analyte concentration was assumed to be only 10 percent higher than the screening value are not presented. The power to detect this small difference was very poor: for 125 of the resulting 140 combinations of n and m, the power was less than 50 percent.

Several observations can be made concerning the data in Table 6-2. **Note:** The statistical power increases as either n (number of replicate composite samples) or m (number of fish per composite) increases. However, greater power is achieved by increasing the number of replicate composite samples as opposed to increasing the number of fish per composite. Furthermore, if the number of replicate composite samples per site and the number of fish per composite are held constant, then, as the ratio of the estimated population variance to the SV increases (i.e., σ/SV), the statistical power decreases.

States may use these tables as a starting point for setting the number of replicate composite samples per site and the number of fish per composite in their fish and shellfish contaminant monitoring studies. The assumption regarding the ratio of the estimated population variance to the SV presented in Section A of Table 6-2 is unrealistic for some fish and shellfish populations. Data in Section B, which reflect more realistic assumptions concerning the estimated population variance, show that States will be able to detect only large differences between the site-specific mean target analyte concentrations and the SV. Specifically, using five replicate composite samples and six to seven fish per composite sample, the power to detect a 50 percent increase over the SV is

Table 6-2. Estimates of Statistical Power of Hypothesis of Interest Under Specified Assumptions

No. of replicate composite samples (n)	Number of fish per composite (m)									
	3	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**12**	**15**
A. Ratio of σ/SV = 0.5 and μ = 1.5 x SV:										
3	6	6	7	8	9	9	9	9	9	9
4	8	9	9	9	9	9	9	9	9	9
5	9	9	9	9	9	9	9	9	9	9
6	9	9	9	9	9	9	9	9	9	9
7	9	9	9	9	9	9	9	9	9	9
10	9	9	9	9	9	9	9	9	9	9
15	9	9	9	9	9	9	9	9	9	9
B. Ratio of σ/SV = 1.0 and μ = 1.5 x SV:										
3	-	-	-	-	-	-	-	-	5	6
4	-	-	-	5	6	6	7	7	8	8
5	-	5	6	7	8	8	8	8	9	9
6	5	6	7	8	8	8	9	9	9	9
7	6	7	8	8	9	9	9	9	9	9
10	8	8	9	9	9	9	9	9	9	9
15	9	9	9	9	9	9	9	9	9	9

-: Power less than 50 percent.
5: Power between 50 and 60 percent.
6: Power between 60 and 70 percent.
7: Power between 70 and 80 percent.
8: Power between 80 and 90 percent
9: Power above 90 percent.

between 70 and 80 percent. However, when the number of fish per composite increases to 8 to 10, the power increases by about 10 percentage points.

One final note on determining the number of replicate composite samples per site and the number of fish per composite should be emphasized. According to Section 6.1.2.3, Phase I intensive studies will focus on those target analytes that exceeded the selected SV used in the screening study. Thus, multiple target analytes may be under investigation during Phase I intensive studies, and the population variances of these analytes are likely to differ. **Note:** States should use the target analyte that exhibits the largest population variance when selecting the number of replicate composite samples per site and the number of fish per composite. This conservative approach supports use of the data in

Section B of Table 6-2 where the ratio of σ/SV is twice that of the data in Section A. States may estimate population variances from historic fish contaminant data or from composite data as described by EPA (1989d). This estimate of σ^2 can be used to determine whether the sampling design (i.e., number of replicate composite samples [n] and number of individuals per composite [m]) should be modified to achieve a desired statistical power.

After States have implemented their fish and shellfish contaminant monitoring program, collected data on cost and variance components, and addressed other design considerations, they may want to consider using an optimal composite sampling protocol as described in Rohlf et al. (1991) for refining their sampling design. An optimal sampling design is desirable because it detects a specified minimum difference between the site-specific mean contaminant concentration and the SV at minimum cost.

6.2 SAMPLE COLLECTION

Sample collection activities should be initiated in the field only after an approved sampling plan has been developed. This section discusses recommended sampling equipment and its use, considerations for ensuring preservation of sample integrity, and field recordkeeping and chain-of-custody procedures associated with sample processing, preservation, and shipping.

6.2.1 Sampling Equipment and Use

In response to the variations in environmental conditions and target species of interest, fisheries biologists have had to devise sampling methods that are intrinsically selective for certain species and sizes of fish and shellfish (Versar, 1982). Although this selectivity can be a hindrance in an investigation of community structure, it is not a problem where tissue contaminant analysis is of concern because tissue contaminant data can best be compared only if factors such as differences in taxa and size are minimized.

Collection methods can be divided into two major categories, active and passive. Each collection method has advantages and disadvantages. Various types of sampling equipment, their use, and their advantages and disadvantages are summarized in Table 6-3 for fish and in Table 6-4 for shellfish. **Note:** Either active or passive collection methods may be used as long as the methods selected result in collection of a representative fish sample of the type consumed by local sport and subsistence fishermen.

A basic checklist of field sampling equipment and supplies is shown in Table 6-5. Safety considerations associated with the use of a boat in sample collection activities are summarized in Table 6-6.

Table 6-3. Summary of Fish Sampling Equipment

Table 6-3 (continued)

Table 6-4. Summary of Shellfish Sampling Equipment

Table 6-4 (continued)

Table 6-5. Checklist of Field Sampling Equipment and Supplies for Fish and Shellfish Contaminant Monitoring Programs

- Boat supplies
  - Fuel supply (primary and auxiliary supply)
  - Spare parts repair kit
  - Life preservers
  - First aid kit (including emergency phone numbers of local hospitals, family contacts for each member of the sampling team)
  - Spare oars
  - Nautical charts of sampling site locations
- Collection equipment (e.g., nets, traps, electroshocking device)
- Recordkeeping/documentation supplies
  - Field logbook
  - Sample request forms
  - Specimen identification labels
  - Chain-of-Custody (COC) Forms and COC tags or labels
  - Indelible pens
- Sample processing equipment and supplies
  - Holding trays
  - Fish measuring board (metric units)
  - Calipers (metric units)
  - Shucking knife
  - Balance to weigh representative specimens for estimating tissue weight (metric units)
  - Aluminum foil (extra heavy duty)
  - Freezer tape
  - String
  - Several sizes of plastic bags for holding individual or composite samples
  - Resealable watertight plastic bags for storage of Field Records, COC Forms, and Sample Request Forms
- Sample preservation and shipping supplies
  - Ice (wet ice, blue ice packets, or dry ice)
  - Ice chests
  - Filament-reinforced tape to seal ice chests for transport to the central processing laboratory

Table 6-6. Safety Considerations for Field Sampling Using a Boat

- Field collection personnel should not be assigned to duty alone in boats.
- Life preservers should be worn at all times by field collection personnel near the water or on board boats.
- If electrofishing is the sampling method used, there must be two shutoff switches--one at the generator and a second on the bow of the boat.
- All deep water sampling should be performed with the aid of an experienced, licensed boat captain.
- All sampling during nondaylight hours, during severe weather conditions, or during periods of high water should be avoided or minimized to ensure the safety of field collection personnel.
- All field collection personnel should be trained in CPR, water safety, boating safety, and first aid procedures for proper response in the event of an accident. Personnel should have local emergency numbers readily available for each sampling trip and know the location of the hospitals or other medical facilities nearest each sampling site.

6.2.1.1 Active Collection—

Active collection methods employ a wide variety of sampling techniques and devices. Devices for fish sampling include electroshocking units, seines, trawls, and angling equipment (hook and line). Rotenone, a chemical piscicide, has been used extensively to stun fish prior to their collection with seines, trawls, or other sampling devices. Rotenone has not been found to interfere with the analysis of the recommended organic target analytes (see Table 4-1) when the recommended analysis procedures are used. See Section 8 for additional information on appropriate analysis methods for the recommended organic target analytes. Devices for shellfish sampling include seines, trawls, mechanical grabs (e.g., pole- or cable-operated grab buckets and tongs), biological and hydraulic dredges, scoops and shovels, rakes, and dip nets. Shellfish can also be collected manually by SCUBA divers. Although active collection requires greater fishing effort, it is usually more efficient than passive collection for covering a large number of sites and catching the relatively small number of individuals needed from each site for tissue analysis (Versar, 1982). Active collection methods are particularly useful in shallow waters (e.g., streams, lake shorelines, and shallow coastal areas of estuaries).

Active collection methods have distinct disadvantages for deep water sampling. They require more field personnel and more expensive equipment than passive collection methods. This disadvantage may be offset by coordinating sampling

efforts with commercial fishing efforts. Purchasing fish and shellfish from commercial fishermen using active collection devices is acceptable; however, field sampling staff should accompany the commercial fishermen during the collection operation to ensure that samples are collected and handled properly and to verify the sampling site location. The field sampling staff then remove the target species directly from the sampling device and ensure that sample collection, processing, and preservation are conducted as prescribed in sample collection protocols, with minimal chance of contamination. This is an excellent method of obtaining specimens of commercially important target species, particularly from the Great Lakes and coastal estuarine areas (Versar, 1982).

More detailed descriptions of active sampling devices and their use are provided in Battelle (1975); Bennett (1970); Gunderson and Ellis (1986); Hayes (1983); Mearns and Allen (1978); Pitt, Wells, and McKone (1981); Puget Sound Estuary Program (1990b); Versar (1982); and Weber (1973).

6.2.1.2 Passive Collection—

Passive collection methods employ a wide array of sampling devices for fish and shellfish, including gill nets, fyke nets, trammel nets, hoop nets, pound nets, and d-traps. Passive collection methods generally require less fishing effort than active methods but are usually less desirable for shallow water sample collection because of the ability of many species to evade these entanglement and entrapment devices. These methods normally yield a much greater catch than would be required for a contaminant monitoring program and are time consuming to deploy. In deep water, however, passive collection methods are generally more efficient than active methods. Crawford and Luoma (1993) caution that passive collection devices (e.g., gill nets) should be checked frequently to ensure that captured fish do not deteriorate prior to removal from the sampling device. Versar (1982, 1984) and Hubert (1983) describe passive sampling devices and their use in more detail.

Purchasing fish and shellfish from commercial fishermen using passive collection methods is acceptable; however, field sampling staff should accompany the fishermen during both the deployment and collection operations to ensure that samples are collected and handled properly and to verify the sampling site location. The field sampling staff can then ensure that sample collection, processing, and preservation are conducted as prescribed in sample collection protocols, with minimal chance of contamination.

6.2.2 Preservation of Sample Integrity

The primary QA consideration in sample collection, processing, preservation, and shipping procedures is the preservation of sample integrity to ensure the accuracy of target analyte analyses. Sample integrity is preserved by prevention of loss of contaminants already present in the tissues and prevention of extraneous tissue contamination (Smith, 1985).

Loss of contaminants already present in fish or shellfish tissues can be prevented in the field by ensuring that the skin on fish specimens has not been lacerated by the sampling gear or that the carapace of crustaceans or shells of bivalves have not been cracked during sample collection resulting in loss of tissues and/or fluids that may contain contaminants. Once the samples have reached the laboratory, further care must be taken during thawing (if specimens are frozen) to ensure that all liquids from the thawed specimens are retained with the tissue sample as appropriate (see Sections 7.2.2, 7.2.3, and 7.2.4).

Sources of extraneous tissue contamination include contamination from sampling gear, grease from ship winches or cables, spilled engine fuel (gasoline or diesel), engine exhaust, dust, ice chests, and ice used for cooling. All potential sources of contamination in the field should be identified and appropriate steps taken to minimize or eliminate them. For example, during sampling, the boat should be positioned so that engine exhausts do not fall on the deck. Ice chests should be scrubbed clean with detergent and rinsed with distilled water after each use to prevent contamination. To avoid contamination from melting ice, samples should be placed in waterproof plastic bags (Stober, 1991). Sampling equipment that has been obviously contaminated by oils, grease, diesel fuel, or gasoline should not be used. All utensils or equipment that will be used directly in handling fish or shellfish (e.g., fish measuring board or calipers) should be cleaned in the laboratory prior to each sampling trip, rinsed in acetone and pesticide-grade hexane, and stored in aluminum foil until use (Versar, 1982). Between sampling sites, the field collection team should clean each measurement device by rinsing it with ambient water and rewrapping it in aluminum foil to prevent contamination.

Note: Ideally, all sample processing (e.g., resections) should be performed at a sample processing facility under cleanroom conditions to reduce the possibility of sample contamination (Schmitt and Finger, 1987; Stober, 1991). However, there may be some situations in which State staff find it necessary to fillet finfish or resect edible turtle or shellfish tissues in the field prior to packaging the samples for shipment to the processing laboratory. This practice should be avoided whenever possible. If States find that filleting fish or resecting other edible tissues must be performed in the field, a clean area should be set up away from sources of diesel exhaust and areas where gasoline, diesel fuel, or grease are used to help reduce the potential for surface and airborne contamination of the samples from PAHs and other contaminants. Use of a mobile laboratory or use of a portable resection table and enclosed hood would provide the best environment for sample processing in the field. General guidance for conducting sample processing under cleanroom conditions is provided in Section 7.2.1. States should review this guidance to ensure that procedures as similar as possible to those recommended for cleanroom processing are followed. If sample processing is conducted in the field, a notation should be made in the field records and on the sample processing record (see Figure 7-2). Procedures for laboratory processing and resection are described in Section 7.2. Procedures for assessing sources of sample contamination through the analyses of field and processing blanks are described in Section 8.3.3.6.

6.2.3 Field Recordkeeping

Thorough documentation of all field sample collection and processing activities is necessary for proper interpretation of field survey results. For fish and shellfish contaminant studies, it is advisable to use preprinted waterproof data forms, indelible ink, and writing implements that can function when wet (Puget Sound Estuary Program, 1990b). When multicopy forms are required, no-carbon-required (NCR) paper is recommended because it allows information to be forwarded on the desired schedule and retained for the project file at the same time.

Four separate preprinted sample tracking forms should be used for each sampling site to document field activities from the time the sample is collected through processing and preservation until the sample is delivered to the processing laboratory. These are

- Field record form
- Sample identification label
- Chain-of-custody (COC) label or tag
- COC form.

6.2.3.1 Field Record Form—

The following information should be included on the field record for each sampling site in both **Tier 1** screening (Figures 6-2 and 6-3) and **Tier 2** intensive studies as appropriate (Figures 6-4 and 6-5):

- Project number
- Sampling date and time (specify convention used, e.g., day/month/year and 24-h clock)
- Sampling site location (including site name and number, county/parish, latitude/longitude, waterbody name/segment number, waterbody type, and site description)
- Sampling depth
- Collection method
- Collectors' names and signatures
- Agency (including telephone number and address)

Figure 6-2. Example of a field record for fish contaminant monitoring program—screening study.

Figure 6-3. Example of a field record for shellfish contaminant monitoring program—screening study.

Figure 6-4. Example of a field record for fish contaminant monitoring program—intensive study.

Figure 6-4 (continued)

Figure 6-5. Example of a field record for shellfish contaminant monitoring program—intensive study.

- Species collected (including species scientific name, composite sample number, individual specimen number, number of individuals per composite sample, number of replicate samples, total length/size [mm], sex [male, female, indeterminate])

Note: States should specify a unique numbering system to track samples for their own fish and shellfish contaminant monitoring programs.

- Percent difference in size between the smallest and largest specimens to be composited (smallest individual length [or size] divided by the largest individual length [or size] x 100; should be ≥75 percent) and mean composite length or size (mm)

- Notes (including visible morphological abnormalities, e.g., fin erosion, skin ulcers, cataracts, skeletal and exoskeletal anomalies, neoplasms, or parasites).

6.2.3.2 Sample Identification Label—

A sample identification label should be completed in indelible ink for each individual fish or shellfish specimen after it is processed to identify each sample uniquely (Figure 6-6). The following information should be included on the sample identification label:

- Species scientific name or code number

- Total length/size of specimen (mm)

- Specimen number

- Sample type: F (fish fillet analysis only)
  S (shellfish edible portion analysis only)
  W (whole fish analysis)
  O (other fish tissue analysis)

- Sampling site—waterbody name and/or identification number

- Sampling date/time (specify convention, e.g., day/month/year and 24-h clock).

A completed sample identification label should be taped to each aluminum-foil-wrapped specimen and the specimen should be placed in a waterproof plastic bag.

6.2.3.3 Chain-of-Custody Label or Tag—

A COC label or tag should be completed in indelible ink for each individual fish specimen. The information to be completed for each fish is shown in Figure 6-7.

Figure 6-6. Example of a sample identification label.

Figure 6-7. Example of a chain-of-custody tag or label.

After all information has been completed, the COC label or tag should be taped or attached with string to the outside of the waterproof plastic bag containing the individual fish sample. Information on the COC label/tag should also be recorded on the COC form (Figure 6-8).

Because of the generally smaller size of shellfish, several individual aluminum-foil-wrapped shellfish specimens (within the same composite sample) may be placed in the same waterproof plastic bag. A COC label or tag should be completed in indelible ink for each shellfish composite sample. If more than 10 individual shellfish are to be composited, several waterproof plastic bags may have to be used for the same composite. It is important not to place too many individual specimens in the same plastic bag to ensure proper preservation during shipping, particularly during summer months. Information on the COC label/tag should also be recorded on the COC form.

6.2.3.4 Chain-of-Custody Form—

A COC form should be completed in indelible ink for each shipping container (e.g., ice chest) used. Information recommended for documentation on the COC form (Figure 6-8) is necessary to track all samples from field collection to receipt at the processing laboratory. In addition, this form can be used for tracking samples through initial laboratory processing (e.g., resection) as described in Section 7.2.

Prior to sealing the ice chest, one copy of the COC form and a copy of the field record sheet should be sealed in a resealable waterproof plastic bag. This plastic bag should be taped to the inside cover of the ice chest so that it is maintained with the samples being tracked. Ice chests should be sealed with reinforced tape for shipment.

6.2.3.5 Field Logbook—

In addition to the four sample tracking forms discussed above, the field collection team should document in a field logbook any additional information on sample collection activities, hydrologic conditions (e.g., tidal stage), weather conditions, boat or equipment operations, or any other unusual activities observed (e.g., dredging) or problems encountered that would be useful to the program manager in evaluating the quality of the fish and shellfish contaminant monitoring data.

6.3 SAMPLE HANDLING

6.3.1 Sample Selection

6.3.1.1 Species Identification—

As soon as fish, shellfish, and turtles are removed from the collection device, they should be identified by species. Nontarget species or specimens of target species that do not meet size requirements (e.g., juveniles) should be returned

Figure 6-8. Example of a chain-of-custody record form.

to the water. Species identification should be conducted only by experienced personnel knowledgeable of the taxonomy of species in the waterbodies included in the contaminant monitoring program. Taxonomic keys, appropriate for the waters being sampled, should be consulted for species identification. Because the objective of both the screening and intensive monitoring studies is to determine the magnitude of contamination in specific fish, shellfish, and turtle species, it is necessary that all individuals used in a composite sample be of a single species. **Note:** Correct species identification is important and different species should never be combined in a single composite sample.

When sufficient numbers of the target species have been identified to make up a composite sample, the species name and all other appropriate information should be recorded on the field record forms (Figures 6-2 through 6-5).

Note: EPA recommends that, when turtles are used as the target species, target analyte concentrations be determined for each turtle rather than for a composite turtle sample.

6.3.1.2 Initial Inspection and Sorting—

Individual fish of the selected target species should be rinsed in ambient water to remove any foreign material from the external surface. Large fish should be stunned by a sharp blow to the base of the skull with a wooden club or metal rod. This club or rod should be used solely for the purpose of stunning fish, and care should be taken to keep it reasonably clean to prevent contamination of the samples (Versar, 1982). Small fish may be placed on ice immediately after capture to stun them, thereby facilitating processing and packaging procedures. Once stunned, individual specimens of the target species should be grouped by species and general size class and placed in clean holding trays to prevent contamination. All fish should be inspected carefully to ensure that their skin and fins have not been damaged by the sampling equipment and damaged specimens should be discarded (Versar, 1982).

Freshwater turtles should be rinsed in ambient water and their external surface scrubbed if necessary to remove any foreign matter from their carapace and limbs. Each turtle should be inspected carefully to ensure that the carapace and extremities have not been damaged by the sampling equipment, and damaged specimens should be discarded (Versar, 1982). Care should be taken when handling large turtles, particularly snapping turtles; many can deliver severe bites. Particularly during procedures that place fingers or hands within striking range of the sharp jaws, covering the turtle's head, neck, and forelimbs with a cloth towel or sack and taping it in place is often sufficient to prevent injury to the field sampling crew (Frye, 1994).

After inspection, each turtle should be placed individually in a heavy burlap sack or canvas bag tied tightly with a strong cord and then placed in an ice-filled cooler. Placing turtles on ice will slow their metabolic rate, making them easier to handle. **Note:** It is recommended that each turtle be analyzed as an individual

sample, especially if the target turtle species is not abundant in the waterbody being sampled or if the collected individuals differ greatly in size or age. Analysis of individual turtles can provide an estimate of the maximum contaminant concentrations to which recreational or substistence fishermen are exposed. Target analyte concentrations in composite samples represent averages for a specific target species population. The use of these values in risk assessment is appropriate if the objective is to estimate the average concentration to which consumers of the target species are exposed over a long period of time. The use of long exposure periods (e.g., 70 years) is typical for the assessment of carcinogenic effects, which may be manifest over an entire lifetime (see Volume II of this guidance series). Noncarcinogenic effects, on the other hand, may cause acute health effects over a relatively short period of time (e.g., hours or days) after consumption. The maximum target analyte contaminant concentration may be more appropriate than the average target analyte concentration for use with noncarginogenic target analytes (U.S. EPA, 1989d). This is especially important for those target analytes for which acute exposures to very high concentrations may be toxic to consumers.

Stone et al. (1980) reported extremely high concentrations of PCBs in various tissues of snapping turtles from a highly contaminated site on the Hudson River. Contaminant analysis of various turtle tissues showed mean PCB levels of 2,991 ppm in fatty tissue, 66 ppm in liver tissue, and 29 ppm in eggs as compared to 4 ppm in skeletal muscle. Clearly, inclusion of the fatty tissue, liver, and eggs with the muscle tissues as part of the edible tissues will increase observed residue concentrations over those detected in muscle tissue only. States interested in using turtles as target species should review Appendix A for additional information on the use of individual samples in contaminant monitoring programs.

Bivalves (oysters, clams, scallops, and mussels) adhering to one another should be separated and scrubbed with a nylon or natural fiber brush to remove any adhering detritus or fouling organisms from the exterior shell surfaces (NOAA, 1987). All bivalves should be inspected carefully to ensure that the shells have not been cracked or damaged by the sampling equipment and damaged specimens should be discarded (Versar, 1982). Crustaceans, including shrimp, crabs, crayfish, and lobsters, should be inspected to ensure that their exoskeletons have not been cracked or damaged during the sampling process, and damaged specimens should be discarded (Versar, 1982). After shellfish have been rinsed, individual specimens should be grouped by target species and placed in clean holding trays to prevent contamination.

A few shellfish specimens may be resected (edible portions removed) to determine wet weight of the edible portions. This will provide an estimate of the number of individuals required to ensure that the recommended sample weight (200 g) is attained. **Note:** Individuals used to determine the wet weight of the edible portion should not be used for target analyte analyses.

6.3.1.3 Length or Size Measurements—

Each fish within the selected target species should be measured to determine total body length (mm). To be consistent with the convention used by most fisheries biologists in the United States, maximum body length should be measured as shown in Figure 6-9. The maximum body length is defined as the length from the anterior-most part of the fish to the tip of the longest caudal fin ray (when the lobes of the caudal fin are compressed dorsoventrally) (Anderson and Gutreuter, 1983).

Each turtle within the selected target species should be measured to determine total carapace length (mm). To be consistent with the convention used by most herpetologists in the United States, carapace length should be measured as shown in Figure 6-9. The maximum carapace length is defined as the straight line distance from the anterior edge of the carapace to the posterior edge of the carapace (Conant and Collins, 1991).

For shellfish, each individual specimen should be measured to determine the appropriate body size (mm). As shown in Figure 6-9, the recommended body measurements differ depending on the type of shellfish being collected. Height is a standard measurement of size for oysters, mussels, clams, scallops, and other bivalve molluscs (Abbott, 1974; Galtsoff, 1964). The height is the distance from the umbo to the anterior (ventral) shell margin. For crabs, the lateral width of the carapace is a standard size measurement (U.S. EPA, 1990c); for shrimp and crayfish, the standard measurement of body size is the length from the rostrum to the tip of the telson (Texas Water Commission, 1990); and for lobsters, two standard measurements of body size are commonly used. For clawed and spiny lobsters, the standard size is the length of the carapace. For spiny lobsters, the length of the tail is also used as a standard size measurement.

6.3.1.4 Sex Determination (Optional)—

An experienced fisheries biologist can often make a preliminary sex determination for fish by visual inspection. The body of the fish should not be dissected in the field to determine sex; sex can be determined through internal examination of the gonads during laboratory processing (Section 7.2.2.4).

An experienced herpetologist can often make a preliminary sex determination of a turtle by visual inspection in the field. The plastron (ventral portion of the carapace) is usually flatter in the female and the tail is less well developed than in the male. The plastron also tends to be more concave in the male (Holmes, 1984). For the common snapping turtle (*Chelydra serpentina*), the cloaca of the female is usually located inside or at the perimeter of the carapace, while the cloaca of the male extends slightly beyond the perimeter of the carapace. The carapace of the turtle should never be resected in the field to determine sex; sex can be determined through internal examination of the gonads during laboratory processing (Section 7.2.3.4.).

[a] Maximum body length is the length from the anterior-most part of the fish to the tip of the longest caudal fin ray (when the lobes of the caudal fin are compressed dorso ventrally (Anderson and Gutreuter, 1983).
[b] Carapace width is the lateral distance across the carapace (from tip of spine to tip of spine) (U.S. EPA, 1990c).
[c] Height is the distance from the umbo to the anterior (ventral) shell margin (Galtsoff, 1964).
[d] Body length is the distance from the tip of the rostrum to the tip of the telson (Texas Water Commission, 1990).
[e] Carapace length is distance from top of rostrum to the posterior margin of the carapace.

Figure 6-9. Recommended measurements of body length and size for fish, shellfish, and turtles.

[e] Carapace length is the distance from the anterior-most edge of the groove between the horns directly above the eyes, to the rear edge of the top part of the carapace as measured along the middorsal line of the back (Laws of Florida Chapter 46-24.003).

[f] Tail length is the distance measured lengthwise along the top middorsal line of the entire tail to the rear-most extremity (this measurement shall be conducted with the tail in a flat straight position with the tip of the tail closed (Laws of Florida Chapter 46-24.003).

[g] Carapace length is the distance from the rear of the eye socket to the posterior margin of the carapace (New York Environmental Conservation Law 13-0329.5.a and Massachusetts General Laws Chapter 130).

[h] Carapace length is the straight-line distance from the anterior margin to the posterior margin of the shell (Conant and Collins, 1991).

Figure 6-9 (continued)

For shellfish, a preliminary sex determination can be made by visual inspection only for crustaceans. Sex cannot be determined in bivalve molluscs without shucking the bivalves and microscopically examining gonadal material. Bivalves should not be shucked in the field to determine sex; sex determination through examination of the gonads can be performed during laboratory processing if desired (Section 7.2.4.2).

6.3.1.5 Morphological Abnormalities (Optional)—

If resources allow, States may wish to consider documenting external gross morphological conditions in fish from contaminated waters. Severely polluted aquatic habitats have been shown to produce a higher frequency of gross pathological disorders than similar, less polluted habitats (Krahn et al., 1986; Malins et al., 1984, 1985; Mix, 1986; Sinderman, 1983; and Sinderman et al., 1980).

Sinderman et al. (1980) reviewed the literature on the relationship of fish pathology to pollution in marine and estuarine environments and identified four gross morphological conditions acceptable for use in monitoring programs:

- Fin erosion
- Skin ulcers
- Skeletal anomalies
- Neoplasms (i.e., tumors).

Fin erosion is the most frequently observed gross morphological abnormality in polluted areas and is found in a variety of fishes (Sinderman, 1983). In demersal fishes, the dorsal and anal fins are most frequently affected; in pelagic fishes, the caudal fin is primarily affected.

Skin ulcers have been found in a variety of fishes from polluted waters and are the second most frequently reported gross abnormality. Prevalence of ulcers generally varies with season and is often associated with organic enrichment (Sinderman, 1983).

Skeletal anomalies include abnormalities of the head, fins, gills, and spinal column (Sinderman, 1983). Skeletal anomalies of the spinal column include fusions, flexures, and vertebral compressions.

Neoplasms or tumors have been found at a higher frequency in a variety of polluted areas throughout the world. The most frequently reported visible tumors are liver tumors, skin tumors (i.e., epidermal papillomas and/or carcinomas), and neurilemmomas (Sinderman, 1983).

The occurrence of fish parasites and other gross morphological abnormalities that are found at a specific site should be noted on the field record form. States interested in documenting morphological abnormalities in fish should review the

protocols for fish pathology studies recommended in the Puget Sound Estuary Program (1990c) and those described by Goede and Barton (1990).

6.3.2 Sample Packaging

6.3.2.1 Fish—

After initial processing to determine species, size, sex, and morphological abnormalities, each fish should be individually wrapped in extra heavy duty aluminum foil. Spines on fish should be sheared to minimize punctures in the aluminum foil packaging (Stober, 1991). The sample identification label shown in Figure 6-6 should be taped to the outside of each aluminum foil package, each individual fish should be placed into a waterproof plastic bag and sealed, and the COC tag or label should be attached to the outside of the plastic bag with string or tape. All of the packaged individual specimens in a composite sample should be kept together (if possible) in one large waterproof plastic bag in the same shipping container (ice chest) for transport. Once packaged, samples should be cooled on ice immediately.

6.3.2.2 Turtles

After inital processing to determine the species, size (carapace length), and sex, each turtle should be placed on ice in a separate burlap or canvas bag and stored on ice for transport to the processing laboratory. A completed sample identification label (Figure 6-6) should be attached with string around the neck or one of the turtle's extremities and the COC tag or label should be attached to the outside of the bag with string or tape. **Note:** Bagging each turtle should not be undertaken until the specimen has been sufficiently cooled to induce a mild state of torpor, thus facilitating processing. The samplers should work rapidly to return each turtle to the ice chest as soon as possible after packaging as the turtle may suddenly awaken as it warms thus becoming a danger to samplers (Frye, 1994). As mentioned in Section 6.3.1, States should analyze turtles individually rather than compositing samples. This is especially important when very few specimens are collected at a sampling site or when specimens of widely varying size/age are collected.

Note: When a large number of individual specimens in the same composite sample are shipped together in the same waterproof plastic bag, the samples must have adequate space in the bag to ensure that contact with ice can occur, thus ensuring proper preservation during shipping. This is especially important when samples are collected during hot weather and/or when the time between field collection and delivery to the processing laboratory approaches the maximum shipping time (Table 6-7).

6.3.2.3 Shellfish—

After initial processing to determine species, size, sex, and morphological abnormalities, each shellfish specimen should be wrapped individually in extra

heavy duty aluminum foil. A completed sample identification label (Figure 6-6) should be taped to the outside of each aluminum foil package. **Note**: Some crustacean species (e.g., blue crabs and spiny lobsters) have sharp spines on their carapace that might puncture the aluminum foil wrapping. Carapace spines should never be sheared off because this would destroy the integrity of the carapace. For such species, one of the following procedures should be used to reduce punctures to the outer foil wrapping:

- Double-wrap the entire specimen in extra heavy duty aluminum foil.
- Place clean cork stoppers over the protruding spines prior to wrapping the specimen in aluminum foil.
- Wrap the spines with multiple layers of foil before wrapping the entire specimen in aluminum foil.

All of the individual aluminum-foil-wrapped shellfish specimens (in the same composite sample) should be placed in the same waterproof plastic bag for transport. In this case, a COC tag or label should be completed for the composite sample and appropriate information recorded on the field record sheet and COC form. The COC label or tag should then be attached to the outside of the plastic bag with string or tape. For composite samples containing more than 10 shellfish specimens or especially large individuals, additional waterproof plastic bags may be required to ensure proper preservation. Once packaged, composite samples should be cooled on ice immediately. **Note**: When a large number of individual specimens in the same composite sample are shipped together in the same waterproof plastic bag, the samples must have adequate space in the bag to ensure that contact with ice can occur; thus ensuring proper preservation during shipping. This is especially important when samples are collected during hot weather and/or when the time between field collection and delivery to the processing laboratory approaches the maximum shipping time (Table 6-7).

6.3.3 Sample Preservation

The type of ice to be used for shipping should be determined by the length of time the samples will be in transit to the processing laboratory and the sample type to be analyzed (Table 6-7).

6.3.3.1 Fish, Turtles, or Shellfish To Be Resected—

Note: Ideally fish, turtles, and shellfish specimens should not be frozen prior to resection if analyses will include edible tissue only because freezing may cause some internal organs to rupture and contaminate fillets or other edible tissues (Stober, 1991; U.S. EPA, 1986b). Wet ice or blue ice (sealed prefrozen ice packets) is recommended as the preservative of choice when the fish fillet, turtle meat, or shellfish edible portions are the primary tissues to be analyzed. Samples shipped on wet or blue ice should be delivered to the processing

Table 6-7. Recommendations for Preservation of Fish, Shellfish, and Turtle Samples from Time of Collection to Delivery at the Processing Laboratory

Sample type	Number per composite	Container	Preservation	Maximum shipping time
Fish[a]				
Whole fish (to be filleted)	3-10	Extra heavy duty aluminum foil wrap of each fish.[b] Each fish is placed in a waterproof plastic bag.	Cool on wet ice or blue ice packets (preferred method) or Freeze on dry ice only if shipping time will exceed 24 hours	24 hours 48 hours
Whole fish	3-10	Same as above.	Cool on wet ice or blue ice packets or Freeze on dry ice	24 hours 48 hours
Shellfish[a]				
Whole shellfish (to be resected for edible tissue)	3-50[c]	Extra heavy duty aluminum foil wrap of each specimen.[b] Shellfish in the same composite sample may be placed in the same waterproof plastic bag.	Cool on wet ice or blue ice packets (preferred method) or Freeze on dry ice if shipping time will exceed 24 hours	24 hours 48 hours
Whole shellfish	3-50[c]	Same as above.	Cool on wet ice or blue ice packets or Freeze on dry ice	24 hours 48 hours
Whole turtles (to be resected for edible tissue)	1[d]	Heavy burlap or canvas bags.	Cool on wet ice or blue ice packets (preferred method) or Freeze on dry ice if shipping time to exceed 24 hours	24 hours 48 hours

[a] Use only individuals that have attained at least legal harvestable or consumable size.
[b] Aluminum foil should not be used for long-term storage of any sample (i.e., whole organisms, fillets, or homogenates) that will be analyzed for metals.
[c] Species and size dependent. For very small shellfish species, more than 50 individuals may be required to achieve the 200-g composite sample mass recommended for screening studies.
[d] Turtles should be analyzed as individual rather than as composite samples.

laboratory within 24 hours (Smith, 1985; U.S. EPA, 1990d). If the shipping time to the processing laboratory will exceed 24 hours, dry ice should be used.

Note: One exception to the use of dry ice for long-term storage is if fish or shellfish are collected as part of extended offshore fieldsurveys. States involved in these types of field surveys may employ shipboard freezers to preserve samples for extended periods rather than using dry ice. Ideally, all fish should be resected in cleanrooms aboard ship prior to freezing.

6.3.3.2 Fish, Turtles, or Shellfish for Whole-Body Analysis—

At some sites, States may deem it necessary to collect fish, turtles, or shellfish for whole-body analysis if a local subpopulation of concern typically consumes whole fish, turtles, or shellfish. If whole fish, turtles, or shellfish samples are to be analyzed, either wet ice, blue ice, or dry ice may be used; however, if the shipping time to the processing laboratory will exceed 24 hours, dry ice should be used.

Dry ice requires special packaging precautions before shipping by aircraft to comply with U.S. Department of Transportation (DOT) regulations. The *Code of Federal Regulations* (49 CFR 173.217) classifies dry ice as Hazard Class 9 UN1845 (Hazardous Material). These regulations specify the amount of dry ice that may be shipped by air transport and the type of packaging required. For each shipment by air exceeding 5 pounds of dry ice per package, advance arrangements must be made with the carrier. Not more than 441 pounds of dry ice may be transported in any one cargo compartment on any aircraft unless the shipper has made special written arrangements with the aircraft operator.

The regulations further specify that the packaging must be designed and constructed to permit the release of carbon dioxide gas to prevent a buildup of pressure that could rupture the package. If samples are transported in a cooler, several vent holes should be drilled to allow carbon dioxide gas to escape. The vents should be near the top of the vertical sides of the cooler, rather than in the cover, to prevent debris from falling into the cooler. Wire screen or cheesecloth should be installed in the vents to keep foreign materials from contaminating the cooler. When the samples are packaged, care should be taken to keep these vents open to prevent the buildup of pressure.

Dry ice is exempted from shipping certification requirements if the amount is less than 441 pounds and the package meets design requirements. The package must be marked "Carbon Dioxide, Solid" or "Dry Ice" with a statement indicating that the material being refrigerated is to be used for diagnostic or treatment purposes (e.g., frozen tissue samples).

6.3.4 Sample Shipping

The fish, turtle, and shellfish samples should be hand-delivered or shipped to the processing laboratory as soon as possible after collection. The time the samples

were collected and time of their arrival at the processing laboratory should be recorded on the COC form (Figure 6-8).

If the sample is to be shipped rather than hand-delivered to the processing laboratory, field collection staff must ensure the samples are packed properly with adequate ice layered between samples so that sample degradation does not occur. In addition, a member of the field collection staff should telephone ahead to the processing laboratory to alert them to the anticipated delivery time of the samples and the name and address of the carrier to be used. Field collection staff should avoid shipping samples for weekend delivery to the processing laboratory unless prior plans for such a delivery have been agreed upon with the processing laboratory staff.